FILE 'MEDLINE, CAPLUS, EMBASE, BIOSIS' ENTERED AT 15:00:36 ON 19 JUL 2003

L1 784 S PROTEIN (1W) SCAFFOLD
L2 365 DUP REM L1 (419 DUPLICATES REMOVED)
L3 47 S L2 AND (CDR OR VARIABLE OR LOOP)
L4 23 S L3 AND PY<2000
L5 0 S L1 AND CTLA!4

L4 ANSWER 9 OF 23 MEDLINE
ACCESSION NUMBER: 95341679 MEDLINE
DOCUMENT NUMBER: 95341679 PubMed ID: 7542349
TITLE: Tendamistat as a scaffold for conformationally constrained phage peptide libraries.
AUTHOR: McConnell S J; Hoess R H
CORPORATE SOURCE: Dupont-Merck Pharmaceutical Company, Experimental Station E328/B33, Wilmington, DE 19880-0328, USA.
SOURCE: JOURNAL OF MOLECULAR BIOLOGY, **(1995 Jul 21)** 250 (4) 460-70.
Journal code: 2985088R. ISSN: 0022-2836.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199508
ENTRY DATE: Entered STN: 19950905
Last Updated on STN: 19960129
Entered Medline: 19950824

AB The alpha-amylase inhibitor Tendamistat (Hoe-467), a 74 amino acid beta-sheet protein from Streptomyces tendae has been expressed on the surface of the filamentous bacteriophage M13. Phage displaying Tendamistat inhibit the hydrolysis of starch by alpha-amylase, indicating that the displayed protein is functional. The displayed Tendamistat has been used as a molecular scaffold for the presentation of constrained random peptides. Two **loops**, comprising residues 38 to 40 and 60 to 65 of Tendamistat, were randomized using PCR mutagenesis. Libraries of approximately 10(8) different mutant Tendamistat molecules were tested for binding to monoclonal antibody A8, which recognizes endothelin. After three cycles of biopanning, phage were isolated that specifically bound the monoclonal antibody. **Loop** swapping and alanine replacement mutagenesis indicated that residues in the 60 to 65 **loop** are responsible for binding to the monoclonal antibody. This work demonstrates the use of relatively small non-antibody **protein scaffolds** for the presentation of constrained random peptide sequences to select for novel binding molecules.

L4 ANSWER 2 OF 23 MEDLINE
ACCESSION NUMBER: 1999327018 MEDLINE
DOCUMENT NUMBER: 99327018 PubMed ID: 10398368
TITLE: Design and expression of soluble CTLA-4 **variable** domain as a scaffold for the display of functional polypeptides.
AUTHOR: Nuttall S D; Rousch M J; Irving R A; Hufton S E; Hoogenboom H R; Hudson P J
CORPORATE SOURCE: CRC for Diagnostic Technologies and CSIRO Molecular Science, Parkville, Victoria, Australia..
Stewart.Nuttall@molsci.csiro.au
SOURCE: PROTEINS, **(1999 Aug 1)** 36 (2) 217-27.
Journal code: 8700181. ISSN: 0887-3585.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199908
ENTRY DATE: Entered STN: 19990816
Last Updated on STN: 19990816
Entered Medline: 19990805

AB We have designed and engineered the human cytotoxic T-lymphocyte associated protein-4 (CTLA-4) **variable** (V-like) domain to produce a human-based **protein scaffold** for peptide display. First, to test whether the CTLA-4 **CDR**-like **loops** were permissive to **loop** replacement/insertion we substituted either the CDR1 or CDR3 **loop** with somatostatin, a 14-residue intra-disulfide-linked neuropeptide. Upon expression as periplasmic-targeted proteins in Escherichia coli, molecules with superior solubility characteristics to the wild-type V-domain were produced. These mutations in CTLA-4 ablated binding to its natural ligands CD80 and CD86, whereas binding to a conformation-dependent anti-CTLA-4 monoclonal antibody showed that the V-domain framework remained correctly folded. Secondly, to develop a system for library selection, we displayed both wild-type and mutated CTLA-4 proteins on the surface of fd-bacteriophage as fusions with the geneIII protein. CTLA-4 displayed on phage bound specifically to immobilized CD80-Ig and CD86-Ig and in one-step panning enriched 5,000 to 2,600-fold respectively over wild-type phage. Bacteriophage displaying CTLA-4 with somatostatin in CDR3 (CTLA-4R-Som3) specifically bound somatostatin receptors on transfected CHO-K1 cells pre-incubated with 1 microg/ml tunicamycin to remove receptor glycosylation. Binding was specific, as 1 microM somatostatin successfully competed with CTLA-4R-Som3. CTLA-4R-Som3 also activated as well as binding preferentially to non-glycosylated receptor subtype Sst4. The ability to substitute **CDR**-like **loops** within CTLA-4 will enable design and construction of more complex libraries of single V-like domain binding molecules. Proteins 1999;36:217-227.